

In re Application of
Roy Duncan

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PATENT
Attorney Docket No. 78973-1C

*cont
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substantially the same as set forth in SEQ ID NO:6, proteins having an amino acid sequence substantially the same as set forth in SEQ ID NO:10, and the like.--

~~Please replace the paragraph beginning at page 7, line 14, with the following rewritten paragraph:~~

a4
--Presently preferred proteins embraced by the above-described profile of properties include proteins having the same amino acid sequence as set forth in SEQ ID NO:2, proteins having the same amino acid sequence as set forth in SEQ ID NO:6, proteins having the same amino acid sequence as set forth in SEQ ID NO:10, and the like.--

~~Please replace the paragraph beginning at page 8, line 7, with the following rewritten paragraph:~~

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--Exemplary proteins embraced by the above-described profile of properties include proteins having an amino acid sequence substantially the same as set forth in SEQ ID NO:10. Presently preferred proteins embraced by the above-described profile of properties include proteins having the same amino acid sequence as set forth in SEQ ID NO:14.--

~~Please replace the paragraph beginning at page 10, line 29, with the following rewritten paragraph:~~

a6
--Exemplary isolated nucleic acids contemplated for use in the practice of the present invention include nucleic acids having a contiguous nucleotide sequence substantially the same as:

nucleotides 25-1607 of SEQ ID NO:1,
nucleotides 25-1607 of SEQ ID NO:5,
nucleotides 27-1579 of SEQ ID NO:9,
nucleotides 25-832 of SEQ ID NO:13, or

variations thereof which encode the same amino acid sequence, but employ different codons for some of the amino acids, or splice variant nucleotide sequences thereof.--

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--The ARV and NBV P11 proteins are small proteins (98 or 95 amino acids, respectively) that share approximately 33% sequence homology and a similar domain organization indicating that these proteins are evolutionarily related (Figure 2). Both proteins lack obvious signal peptides, suggesting that they insert in membranes post-translationally. Both proteins also contain one predicted transmembrane domain located in the central portion of the protein resulting in small (approximately 40 amino acid) intracellular and extracellular domains. The conserved clustering of positively charged amino acids on the carboxy-proximal side of the transmembrane domain is consistent with the amino-terminal domain residing extracellularly (von Heijne, *Curr. Op. Cell Biol.*, 2:604-608 (1990)). The four cysteine residues in each protein are conserved, suggesting that the ARV and NBV P11 proteins assume a similar tertiary and quaternary structure. The ARV P11 protein is devoid of N-linked glycosylation sites, implying that post-translational glycosylation is not required for functional protein folding, a prediction that has been confirmed experimentally (see Duncan et al. (1996), *supra*). Although the NBV P11 protein contains a single potential N-linked glycosylation site, this site is probably not glycosylated since inhibitors of glycosylation fail to affect NBV-induced cell fusion (see Wilcox and Compans (1983)). The size, absence of signal peptides, and N-linked glycosylation, and predicted domain organization of the ARV and NBV P11 proteins clearly distinguishes these proteins from the well characterized enveloped virus fusion proteins and suggests that P11 represents a novel type of membrane fusion protein.--

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Please replace the paragraph beginning at page 33, line 24, with the following rewritten paragraph:

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--The fusion-inducing potential of these reovirus proteins has been directly demonstrated by expressing them in transfected cells in the absence of any other reovirus proteins; intracellular expression triggers the induction of cell-cell fusion and syncytium formation characteristic of virus infection by this group of fusogenic reoviruses. Thus, quail cell monolayers were mock transfected, or transfected with plasmid DNA expressing the ARV, BRV, or NBV fusion proteins. Transfected cells were fixed and the nuclei stained using a Wright-Giemsa stain at 36 hr post infection and the stained monolayers were photographed at 100x magnification.--

Please replace the paragraph beginning at page 11, line 11, with the following rewritten paragraph:

--Presently preferred isolated and purified nucleic acids, or functional fragments thereof contemplated according to the invention are nucleic acids encoding the above-described proteins, e.g.,

(a) DNA encoding the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:10 or SEQ ID NO:14, or

(b) DNA that hybridizes to the DNA of (a) under moderately stringent conditions, wherein said DNA encodes biologically active fusion protein, or

(c) DNA degenerate with respect to either (a) or (b) above, wherein said DNA encodes biologically active fusion protein.--

Please replace the paragraph beginning at page 11, line 24, with the following rewritten paragraph:

--As employed herein, the term "contiguous nucleotide sequence substantially the same as" refers to DNA having sufficient homology to the reference polynucleotide, such that it will hybridize to the reference nucleotide under typical stringency conditions employed by those of skill in the art. In one embodiment, DNA having substantially the same nucleotide sequence as the reference nucleotide encodes substantially the same amino acid sequence of SEQ ID NOS:2, 6, 10 or 14. In another embodiment, DNA having "a contiguous nucleotide sequence substantially the same as" has at least 60% homology with respect to the nucleotide sequence of the reference DNA fragment with which the subject DNA is being compared. In a preferred embodiment, the DNA has at least 70%, more preferably 80%, homology to the comparative nucleotide sequence; with greater than about 90% homology being especially preferred.--

Please replace the paragraph beginning at page 14, line 8, with the following rewritten paragraph:

--As used herein, a nucleic acid "probe" is single-stranded DNA or RNA, or analogues thereof, that has a sequence of nucleotides that includes at least 14, preferably at least 20, more preferably at least 50, contiguous bases that

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are the same as (or the complement of) any 14 or more contiguous bases set forth in any of SEQ ID NOS:1, 5, 9 or 13. Probes may be labeled by methods well-known in the art, as described hereinafter, and used in various diagnostic kits.--

Q10
Please replace the paragraph beginning at page 23, line 6, with the following rewritten paragraph:

--The detergent-protein complexes can be mixed with lipids and the detergent removed by dialysis, chromatography, or extraction according to standard published procedures, similar to methods used to generate influenza HA or Sendai virus F protein-containing virosomes (see Grimaldi, *Res. Virol.*, 146:289-293 (1995) and Ramani et al., *FEBS Lett.*, 404:164-168 (1997)). These procedures will result in the production of proteoliposomes, lipid vesicles containing the ARV, NBV, or BRV fusion proteins embedded in the vesicle membrane. Once again, optimal conditions for proteoliposome production can be empirically determined as can the lipid composition and size of the proteoliposomes which can affect the efficiency of liposome-cell fusion. Bioactive molecules of interest (e.g., nucleic acids, proteins or peptides, pharmacological compounds, and the like) can be included during the formation of the proteoliposomes to facilitate packaging of the molecule within the liposomes. The proteoliposomes can be purified by centrifugation and used to deliver bioactive molecules intracellularly, either in cell culture or *in vivo*, by protein-enhanced fusion of the proteoliposomes with cell membranes.--

A11
Please replace the paragraph beginning at page 27, line 13, with the following rewritten paragraph:

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--The two strains of ARV were grown in monolayers of QM5 cells, a continuous quail cell line (see Antin and Ordahl, *Devel. Biol.*, 143:111-121 (1991)) while the fusogenic mammalian reoviruses were grown in monkey Vero cells. Virus particles were isolated and concentrated from infected cell lysates by differential centrifugation, as previously described (see Duncan, *Virology*, 219:179-189 (1996)).--

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Please replace the paragraph beginning at page 28, line 28, with the following rewritten paragraph:

--The ARV and NBV S1 cDNA clones and the BRV S4 cDNA clone were subcloned into the eukaryotic expression vector pcDNA3 (Invitrogen) under the

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control of the CMV promoter. Plasmid DNA was isolated and purified on Qiagen midi columns (Qiagen) according to the manufacturer's specifications. Plasmid DNA (1 μ g) was mixed with Lipofectamine (3 μ l) (Life Technologies Inc.) and used to transfect sub-confluent cell monolayers grown in 12 well cluster plates. Transfected cell monolayers were incubated at 37°C for 24-48 hr before being fixed with methanol and stained using a water-soluble Wright-Giemsa stain (DiffQuik; VWR-Canlab) or by immunostaining using viral-specific antiserum obtained from infected animals, as previously described (see Duncan et al., *Virology*, 224:453-464 (1996)). Cell fusion was assessed by light microscopy of stained monolayers and syncytial foci were photographed at 100x magnification.--

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Please replace the paragraph beginning at page 29, line 17, with the following rewritten paragraph:

--Sequence analysis determined that the ARV and NBV S1 genome segments contained three sequential overlapping open reading frames (ORFs) while the BRV S4 genome segment contained 2 ORFs. In order to determine which ORF encoded the viral fusion protein, portions of these genome segments were subcloned into pcDNA3 by PCR amplification of individual regions using sequence-specific primers as indicated in the figures. The subcloned regions were analyzed for their fusion-inducing ability by transfection analysis as described above.--

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Please replace the paragraph beginning at page 29, line 29, with the following rewritten paragraph:

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--Two unrelated fusion proteins responsible for the cell-cell fusion induced by avian reovirus (ARV) and the only two fusogenic mammalian reoviruses, Nelson Bay virus (NBV) and baboon reovirus (BRV) have been identified. These proteins are referred to herein as P11 (for ARV and NBV) and P15 (for BRV) to reflect their approximate predicted molecular weights. The genes encoding P11 from two strains of ARV (strain 176 and strain 138) and from NBV have been cloned and sequenced, as has the gene from BRV that encodes P15. The sequence-predicted structural organization of these proteins has been analyzed, and the membrane fusion properties thereof have been directly demonstrated.--

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Please replace the paragraph beginning at page 32, line 6, with the following rewritten paragraph: